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SI: HETEROLOGOUS GENE EXPRESSION

Expression of the hepatitis B virus surface antigen in Drosophila S2 cells

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Abstract *Drosophila melanogaster* S2 cells were transfected with a plasmid vector (pAcHBsAgHy) containing the S gene, coding for the hepatitis B virus surface antigen (HBsAg), under control of the constitutive drosophila actin promoter (pAc), and the hygromycin B (Hy) selection gene. The vector was introduced into Schneider 2 (S2) Drosophila cells by DNA transfection and a cell population (S2AcHBsAgHy) was selected by its resistance to hygromycin B. The pAcHBsAgHy vector integrated in transfected S2 cell genome and approximately 1,000 copies per cell were found in a higher HBsAg producer cell subpopulation. The HBsAg production varied in different subpopulations, but did not when a given subpopulation was cultivated in different culture flasks. Higher HBsAg expression was found in S2AcHBsAgHy cells cultivated in Insect Xpress medium (13.5 μg/1E7 cells) and SFX medium (7 μg/1E7 cells) in comparison to SF900II medium (0.6 μg/1E7 cells). An increase of HBsAg was observed in culture maintained under hygromycin selection pressure. Data presented in the paper show that S2AcHBsAgHy cells produce efficiently the HBsAg which is mainly found in the cell supernatant, suggesting that HBsAg is secreted from the cells. The data also show that our approach using the Drosophila expression system is suitable for the preparation of other viral protein preparation.

Keywords Drosophila expression system · HBsAg · S2 cells

Abbreviation
HBsAg Hepatitis B virus surface antigen

Introduction

Hepatitis B virus (HBV) is a major etiological agent of worldwide human diseases, including hepatitis, cirrhosis and hepatocellular carcinoma. There are approximately 2 billion acute infected people and 360 million chronic infections in the world at serious risk of developing chronic liver disease and, possibly, primary liver cancer (Valsamakis 2007).

Hepatitis B control depends largely on the disposal of a safe, effective and cheap vaccine. The major viral envelope protein, HBV surface antigen (HBsAg), induces a protective immune response during infection (Tiollais et al. 1981). Currently, native or recombinant Hepatitis B virus surface antigen (HBsAg) is the sole approved component for human vaccines against

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hepatitis B (McAleer et al. 1984). The HBsAg vaccination induces both cellular and humoral responses and appears as an attractive immunization system against viral infections (Zhao et al. 2000).

The HBsAg is the viral envelope and it is composed by 3 proteins S (small), M (medium) and L (large) codified by only one ORF. The S protein is more abundantly found in HBsAg. The large scale expression of the S protein is the basis for the production of a less expensive vaccine against HBV. A yeast derived recombinant HBsAg has been commercially available since 1987. This system consists exclusively of the expression of the S gene, which codes for HBsAg (McAleer et al. 1984; Valenzuela et al. 1982; Miyanohara et al. 1983). Several other systems have been described, employing cultured plant, mammalian and insect cells carrying the S gene (Ganapathi et al. 2007; Lou et al. 2007; Carloni et al. 1984; Chen et al. 1991; Stephenne 1989). In contrast to yeast systems, HBsAg from animal cells is normally secreted as a component of 22 nm particles, allowing easy protein purification.

Drosophila melanogaster Schneider 2 (S2) cells have become increasingly utilized over the past few years for the expression of heterologous proteins (Guy 1997). High levels of protein expression with pharmacological and biotechnological interest can be achieved using Drosophila Expression System (DES) procedure with a plasmid encoding a gene of interest (Invitrogen). After 3 weeks of selection, this system using Drosophila promoters is able to generate a stable polyclonal cell line processing up 1,000 gene copies per cell (Kirkpatrick et al. 1995). Proteins of prokaryotic, eukaryotic and viral origin have been expressed in this system showing to be appropriately processed and biologically active (Angelichio et al. 1991; Culp et al. 1991; Li et al. 1996; Deml et al. 1999; Nilsen and Castellino 1999; Lee et al. 2000; Hill et al. 2001; Yokomizo et al. 2007; Santos et al. 2007). The HBsAg expression by S2 cells transfected or co-transfected with expression and selection vectors was observed by Deml et al. (1999) in an inductive system using methotrexate selection. In our expression system a vector with a promoter (actin) enabling a constitutive expression was used and comparable levels of HBsAg expression were found. Based on these evidences DES has an important potential as a gene expression system.

We were interested in measuring the efficiency of a constitutive expression vector, which integrates in Drosophila cells (10–1,000 copies per cell). For this purpose we used the HBV S gene, that has been studied in different expression systems (yeast, bacteria, animal and plant cells). The HBV S gene, under the control of the drosophila actin promoter (pAc), was inserted in a plasmid carrying the hygromycin B resistance gene. The expression of HBsAg in cells harboring the vector was analyzed in different culture conditions, such as culture flasks and medium. HBsAg produced in these cells, which is comparable to what is described for other animal cell systems, was found both in cell supernatant and cell lysate suggesting that the HBsAg is secreted by the cells.

Materials and methods

Cell line and plasmid vector

Drosophila melanogaster Schneider 2 cells (S2) (Schneider 1972) were maintained at 28 °C under normal atmosphere in T-25 flasks in SF900II serum free medium (Invitrogen).

The plasmid vector pAcHBsAgHy is shown in Fig. 1. It was constructed by insertion of a 1.3 Kpb *Bam*HI fragment with the HBV S gene and a 2.7 Kpb *Pvu*II fragment with the hygromycin resistance gene under control of the Drosophila copia promoter into the

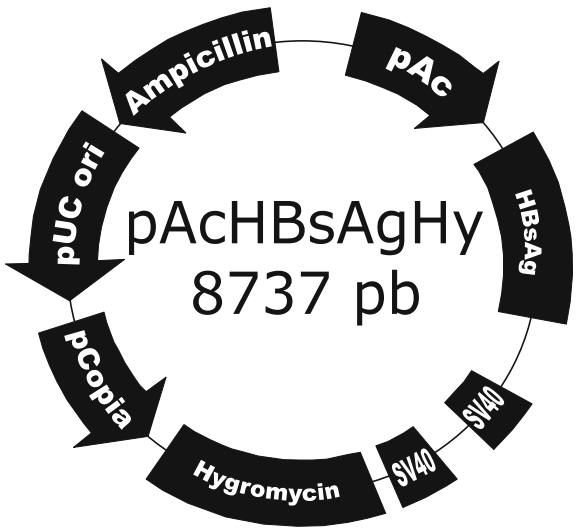

Fig. 1 pAcHBsAgHy vector map. pAc represents the drosophila actin promoter; HBsAg, the gene of the surface antigen of Hepatitis B virus; SV40, the signal of polyadenylation of the SV40 virus; pCopia, the promoter of drosophila copia gene. Hygromycin, the hygromycin resistance gene; pUC ori and ampicillin, represent the replication origin and selection gene in bacteria, respectively

pAc5,1-V5 HisA vector (Invitrogen). The plasmid pAcHBsAgHy also contains the pUC origin of DNA replication and a gene for ampicillin selection in bacteria and a sequence of C-terminal peptide containing V5 epitope and a polyhistidine (6× His) tag. The S gene was placed under control of the constitutive drosophila actin promoter and its orientation was confirmed by restriction mapping and sequencing.

DNA transfection

The S2 cell line transfection with 2 μg of vector DNA (pAcHBsAgHy) was carried out on the basis of the lipid-mediated procedure, using cellfectin reagent (Invitrogen). Hygromycin B (600 μg/mL) was added to the medium for selection 3 days after transfection. The resistant cells (S2AcHBsAgHy) were pooled or subcloned independently for further studies. After selection, the cells were maintained in hygromycin free medium (SF900II-Invitrogen).

S2AcHBsAg cell culture

We selected S2AcHBsAgHy cell subpopulations by limiting dilution in cell cultures in 96 well microplates. 1E6, 1E5 and 1E4 cells were plated in 200 μL of SF900II medium and the HBsAg expression was individually measured. Cell subpopulations (9C, 8A, 8C and 12E) with high HBsAg expression were then further cultivated.

For the study of culture system, S2AcHBsAgHy 9C subpopulation cells were cultivated in different flasks culture. Static cultures: T25 and T75 flasks with, respectively, 5 and 15 mL of SF900II medium. Agitated cultures: shake bottles of 100 mL and 250 mL of total volume with working volumes of respectively 15 mL and 30 mL of SF900II medium at 100 rpm in orbital shaker (unless otherwise indicated). Viable cell concentration was measured by trypan blue exclusion method. Glucose, lactate and glutamine concentrations were measured in a YSI-2700 biochemical analyzer (Yellow Spring Instruments).

For the study of medium influence, S2AcHBsAHy cell subpopulations were adapted in respective media for 4 passages in shake flasks at an initial cell seeding of 5 × 1E5 cells per mL and then were cultivated in 100 mL shake flasks in a working volume of 15 mL of the following different media: InsectXpress (BioWhittaker), DES (Invitrogen), SFX (HyClone), Schneider (Invitrogen) + 10% FBS, SF900-II (Invitrogen) or TC-100 (Gibco) + 10% FBS.

HBsAg quantification

Culture samples of S2AcHBsAgHy were centrifuged (1 krpm/5 min) for cell and supernatant fractions separation. The cell fraction was treated with lysis buffer (25 mM Tris, 25 mM NaCl, 5 mM $MgCl_2$ and 0.2% Nonidet p-40) for 10 min, as already described (Astray et al. 2008). The presence of HBsAg in both fractions was evaluated by enzyme linked immunosorbent assay (ELISA), using Hepanostika HBsAg Uni-Form II (Biomerieux) according to the instructions of the manufacturer. The amount of protein was determined by comparison of HBsAg values with serial dilutions of known concentrations of standard HBsAg (Hepatitis Section of the Instituto Butantan). Total HBsAg expression, expressed in μg/1E7 cell, was obtained by the sum of values obtained for the cell supernatant and cell lysate.

Southern blotting

Total DNA was extracted from transfected cells using Triton X lysis method (Sambrook et al. 1989). Briefly, cells were harvested and lysed by a Triton X100 and sodium dodecyl sulfate (SDS) solution. The culture dish was rocked gently and the cell lysate was scraped into centrifuge tube. The DNA solution was purified by chloroform extractions and pelleted with ethanol. The plasmid was resuspended in TRIS-EDTA buffer and analysed by electrophoresis in a 0.5% agarose gel. After Southern transfer, DNA was detected using linear pAcHBsAgHy DNA probe with alkaline phosphatase (Amersham Biosciences). Intensity of bands from the autoradiograms was determined by densitometry analysis employing a gel documentation system and the Alpha Digidoc software (Alpha Digidoc, Life Technologies). The values obtained were used to calculate the number of HBsAg S DNA copies per cell.

Results

HBsAg expression by S2AcHBsAgHy cell population

We have quantified the HBsAg expression in cell lysates as well as in cell supernatants by ELISA

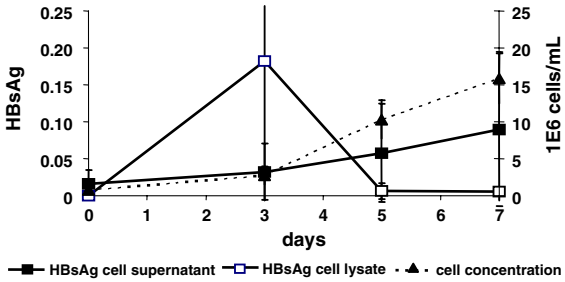

Fig. 2 Cell growth and HBsAg concentration cell supernatants and cell lysates. The S2AcHBsAgHy cells were cultivated in 100 mL shake flasks for 7 days with SF900II medium at 28 °C and 100 rpm. Periodically samples were taken, the cells counted and the HBsAg evaluated in the supernatant (μg/mL) and in cell fraction (μg/1E7 cells) after lysis with buffer. Data of at least 4 different experiments and standard deviation are shown

assay. As shown in Fig. 2, the cells grow until day 7 reaching a concentration of 1.7 × 1E7 cells/mL. The S2AcHBsAgHy cell population was capable of synthesizing the HBsAg, which was detected in cell lysates attaining a concentration of 0.19 μg/1E7 cells at day 3 and in cell supernatant attaining 0.10 μg/mL at day 7. After 3 days of culture, most of the HBsAg expressed by S2AcHBsAgHy cells was found in the cell supernatants, indicating the protein secretion.

Selection of S2AcHBsAgHy subpopulations

We select cell subpopulations with best capacity of synthesizing the HBsAg by limiting dilution in S2AcHBsAgHy cell cultures in 96-well microplates. After establishment of selected cell subpopulations, we analyzed their ability to produce HBsAg by measuring the total amount of HBsAg produced (value of cell supernatant plus the value in cell lysates) by ELISA as well as by evaluating the HBsAg S DNA content by southern-blotting. As shown in Fig. 3b, the S2AcHBsAgHy cell subpopulations, at day 6 of culture, were capable of producing HBsAg ranging from 0.1–0.6 μg/1E7 cells, when the original S2AcHBsAgHy was capable of producing 0.3 μg/1E7 cells). In S2AcHBsAgHy 9C cell cultures the level of HBsAg production reached 0.6 μg/1E7 cells.

In order to evaluate the amount of HBsAg S DNA present in the S2AcHBsAgHy cells and in their subpopulations, cellular DNA was extracted, separated on a 1% agarose gel and analyzed by Southern

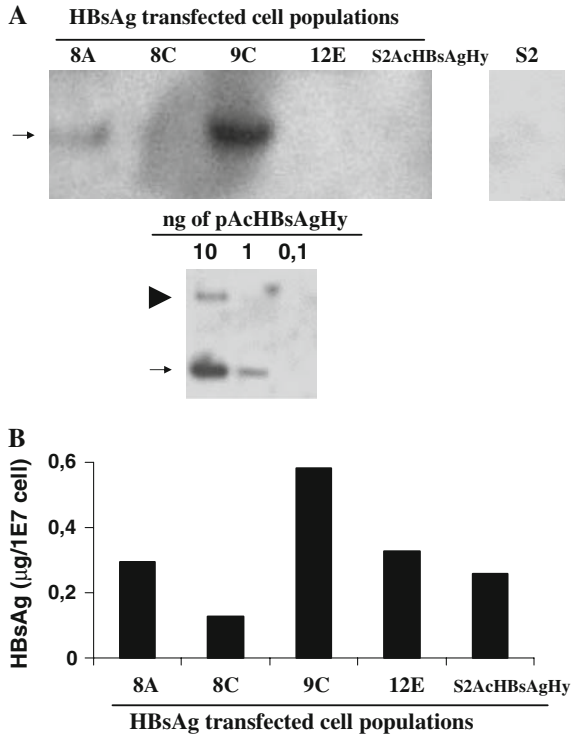

Fig. 3 Southern blotting analysis (**a**) and total HBsAg expression (**b**) of S2AcHBsAgHy cell populations. S2AcHBsAgHy cells were cultivated in shake flasks with SF900II for 6 days at 28 °C and 100 rpm, when cell supernatants and cell fractions were collected. Cell fraction was treated with lysis buffer for 10 min HBsAg DNA was evaluated by Southern blotting analysis (**a**) of DNA extracted from transfected cell populations or from pAcHBsAgHy. Arrows indicate the supercoiled pAcHBsAgHy DNA and the arrowhead the circular pAcHBsAgHy DNA. Total HBsAg content in cell supernatants and cell fractions was evaluated by ELISA (**b**) and expressed in μg/1E7 cells. The total HBsAg was obtained by the addition of values obtained for the cell supernatant and cell lysate. S2AcHBsAgHy is the initial cell population and 8A, 8C, 9C and 12E are selected subpopulations

blotting (Fig. 3a). The expected >10 Kpb bands were found indicating that the vector was associated with the cell genome. The intensities of DNA bands shown in Fig. 3a were quantified by densitometry, and the values compared with those from known concentrations of pAcHBsAgHy S DNA, allowing an estimation of the number of copies of HBsAg S DNA/cell. The higher value found was in S2AcHBsAgHy 9C subpopulation (∼1,000 copies of HBsAg S DNA/cell). In S2AcHBsAgHy 8A subpopulation were found approximately 100 copies of HBsAg S DNA/cell. In other subpopulations and in the original population less than 10 copies of HBsAg

S DNA/cell, which was the lowest detectable level in Southern blotting assays of HBsAg S DNA/cell were present. The ELISA and Southern blotting study of different S2HBsAgHy populations suggested a direct correlation between the HBsAg expression and the number of HBsAg S DNA copies integrated in the cellular genome.

HBsAg expression of S2AcHBsAg 9C cells cultivated in different culture flasks

S2AcHBsAgHy 9C cells were cultivated in SF900II medium using different culture systems: T25 and T75 flasks, 100 and 250 shake flasks under orbital agitation at 100 rpm. The cell growth, the HBsAg expression in cell supernatants and cell lysates and the cell metabolism were measured. As shown in Fig. 4, when cultivated in different systems, S2AcHBsAgHy 9C cells expressed the HBsAg and the cultures in 100 mL shaker bottle attained a cell density of 1.5 × 1E7 cell/mL at day 7 (Fig. 4a). During the culture, glucose was progressively consumed in all cultures, attaining low glucose concentration at day 11 coinciding with a viable cell concentration decrease (Fig. 4b). Glutamine was also consumed but still present in relatively high concentrations at the end of cultures (Fig. 4c). Lactate was present in cultures in low concentrations (Fig. 4d). The HBsAg expression could be detected in cell lysates and in cell supernatants (Fig. 4e and f). Similar concentration of HBsAg was found in supernatants of all cultures.

HBsAg expression of S2AcHBsAg 9C cells cultivated in different media

The kinetics of total HBsAg expression per 1E7 cells was evaluated in S2AcHBsAgHy 9C cells cultivated for 11 days in different culture media in shake flasks. As shown in Fig. 5, S2AcHBsAgHy 9C cells cultivated in Insect Xpress and SFX led to high HBsAg expression per 1E7 cells. In contrast, when they were cultivated in SF900II, TC100 + 10% FCS and DES media a reduced HBsAg expression per 1E7 cells was

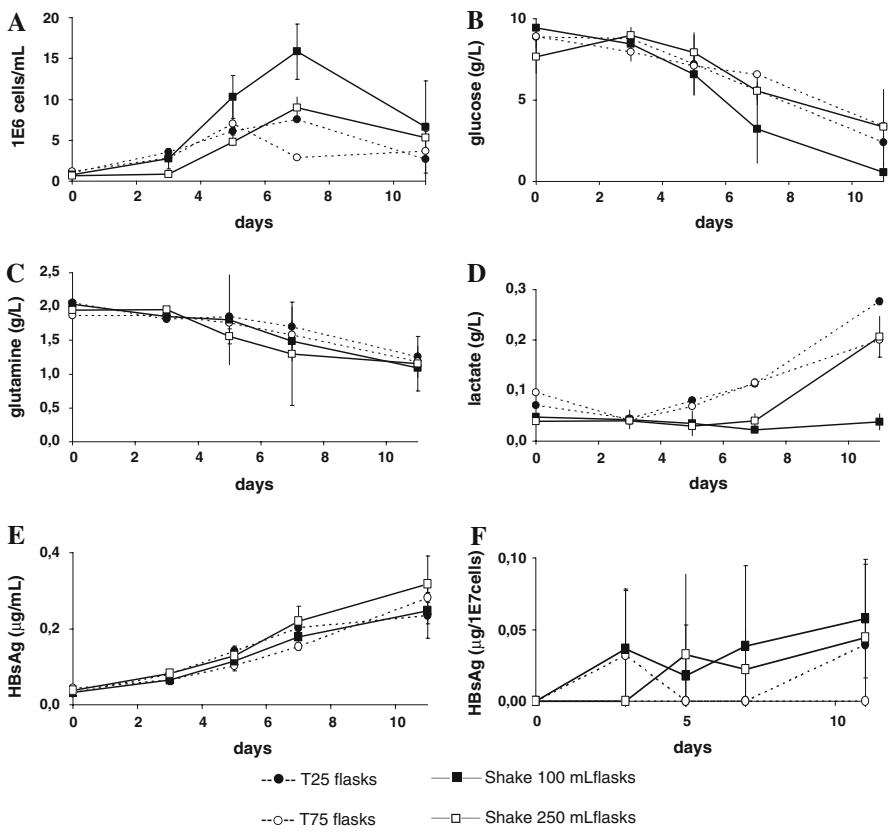

Fig. 4 Cell growth (**a**), glucose (**b**) and glutamine (**c**) consumption, lactate production (**d**) and HBsAg expression (**e**, **f**) of S2AcHBsAgHy 9C cells cultivated in T-flasks or shake flasks. Cells were cultivated for 11 days with SF900II culture medium at 28 °C and 100 rpm. Periodically samples were taken and the cell growth, glucose, glutamine and lactate concentrations were evaluated in these cultures. HBsAg expression in S2AcHBsAgHy 9C cell supernatant and cell lysate were measured. Data of at least 3 different experiments and standard deviation are shown

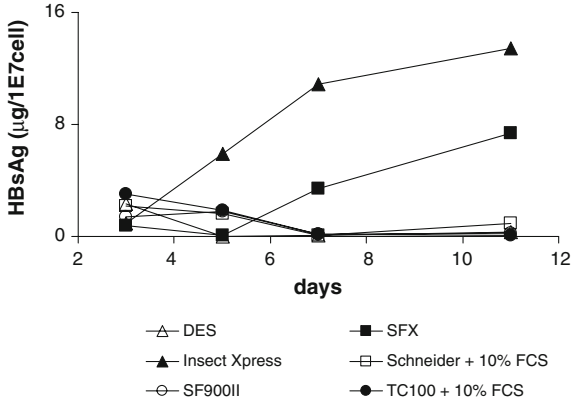

Fig. 5 Kinetics of total HBsAg expression by S2HBsAgHy 9C cells cultivated in different media. S2AcHBsAgHy 9C cells were adapted and then cultivated in the indicated media, in shake flasks for 11 days at 28 °C and 100 rpm, when cells and cell supernatants were collected. Cells were then treated with lysis buffer for 10 min and the HBsAg content evaluated by ELISA. Total HBsAg expression, expressed in µg/1E7 cell, was obtained by the sum of values obtained for the cell supernatant and cell lysate. Data of a representative experiment are shown

observed. S2AcHBsAgHy 9C cells cultivated in Schneider medium + 10% FCS showed both a reduced cell growth and HBsAg expression per 1E7 cells. The total values of HBsAg production in these cultures where of 115, 12, 5, 4.5, 2 and 0.3 µg for cultures in, respectively, SFX, Insect Xpress, Schneider + 10% FCS, SF900II, DES and TC100 medium + 10% FCS. The discrepancy of the total and per cell HBsAg productivity is explained by the high or low ability of S2AcHBsAgHy 9C cells to grow in the respective media. SFX and Insect Xpress media showed a good performance of cell growth and HBsAg synthesis leading to high values of both, total or per cell HBsAg productivity.

HBsAg expression of S2AcHBsAg 9C cells in cultures with or without selective hygromycin pressure

The effect of hygromycin selective pressure on total HBsAg expression by S2AcHBsAgHy 9C cell subpopulation was analyzed. After 15 passages without the hygromycin, S2AcHBsAgHy 9C cells were cultured with 300 µg/mL of hygromycin for further 10 passages. As shown in Table 1, hygromycin selective pressure enhanced the HBsAg expression of S2AcHBsAgHy 9C cells.

Table 1 Total HBsAg expression by S2AcHBsAgHy 9C cell subpopulation cultured with or without hygromycin selection pressure

	HBsAg (µg/mL)
Without hygromycin	0.06 ± 0.02
With hygromycin	0.10 ± 0.02

A cell population was first cultured for 15 passages in absence of hygromycin and then for 10 passages again in presence of hygromycin. Another cell population was always maintained in absence of hygromycin. Both cell populations were then cultivated in 100 mL shake flasks for 11 days at 28 °C and 100 rpm when cells and cell supernatants were collected. Cells were then treated with lysis buffer for 10 min and the HBsAg content evaluated by ELISA. Total HBsAg expression, expressed in µg/mL, was obtained by the sum of values obtained for the cell supernatant and cell lysate. Significant for $p < 0.01$

Discussion

This work describes the establishment of Drosophila cell lines carrying vectors that express and secrete the HBsAg stably in the cell medium, suggesting that it is liberated from the cells as a component of the 22-nm particle, as it has been observed by others (Deml et al. 1999). Kinetic studies of HBsAg synthesis by ELISA showed that S2AcHBsAgHy 9C cell subpopulation was capable of synthesizing the heterologous protein, which could be found in cell lysates and supernatants (Fig. 2). The HBsAg synthesis paralleled the cell multiplication and was found in cell lysates and supernatants.

Upon transfection cell subpopulations, characterized by efficient and stable recombinant gene expression, can be selected. This can be achieved by selecting cell populations resistant to an antibiotic marker and/or by using limiting dilution procedure. In view of the difficulties to grow S2 cells at low densities the second approach is not easily applicable. Even so we could obtain S2AcHBsAgHy subpopulations (Fig. 3) and one of them, (S2AcHBsAgHy 9C) was shown to express 3× more HBsAg than the initial transfected population. The S2AcHBsAgHy 9C subpopulation showed approximately 1,000 copies of HBsAg S DNA/cell, indicating a direct correlation of recombinant gene expression with the number of vector copies. A high heterogeneity of HBsAg production among different selected S2AcHBsAgHy subpopulations could be explained by the selection of clones harboring plasmids with rearrangements, since transfection of plasmid DNA into a cell

culture leads to a high frequency of genetic instability (Calos et al. 1983; Razzaque et al. 1983). Since the expression of an heterologous transfected gene, in the cell context, depends on its properly insertion into the cell genome, it is conceivable that a variable number of gene copies, upon cell transfection and gene integration, became inserted in untranscribed regions of the genome. Even efficient procedures of gene transfections may lead to integrated gene copies that are not expressed. Sodium butyrate (NaBu), a histone deacetylase inhibitor, affecting the chromatin structure and leading to the destabilization of nucleosomal structure, exposing the chromatin may facilitate binding of transcription factors to DNA resulting in an increased and deregulated cell protein synthesis. We have observed that S2AcHBsAgHy 9C cell treatment with NaBu led to a decreased cell multiplication and decreased viability, but was not accompanied by an increase of HBsAg expression (data not shown).

The HBsAg expression was analyzed when cells were cultivated in different media (Fig. 5). The S2AcHBsAgHy cells cultured in Insect Xpress medium were capable to produced up to 13.5 μg of HBsAg/1E7 cells, in contrast to 0.2 μg of HBsAg/1E7 cell produced by S2AcHBsAgHy cultured in SF900II. Nevertheless, although a higher HBsAg expression per cell was observed in Insect Xpress cultures, the cultures performed in SFX medium were capable of producing a much higher total amount of HBsAg (115 μg of HBsAg) when compared to the values obtained with Insect Xpress cultures (12 μg of HBsAg). These data suggest that the culture medium composition plays an important role on the gene expression and its relationship with cell growth. We should mention that the composition of these commercial media (SF900II, SFX and Insect Xpress) is not known. They present serum free media optimized for the growth and maintenance of insect cells and for large-scale production of recombinant proteins.

Cell metabolism and HBsAg expression were studied by culturing the cells in agitated or static flasks (Fig. 4). No significant differences were observed in terms of cell metabolism or HBsAg expression. Glutamine and glucose consumption or lactate production as well as cell growth or HBsAg expression were comparable in these different systems. In general the HBsAg was first detected in cell lysates and then, from day 5 on in increasing concentrations in supernatants (Fig. 2). It may indicate a higher production in early stages of culture, increased degradation or variable efficiency in the secretion process during the culture. The systems used for cell cultivation did not interfere significantly in the HBsAg expression, indicating low constraints for scaling up (Fig. 4).

The HBsAg expression of S2AcHBsAgHy 9C cultivated with hygromycin selective pressure for 10–15 passages was evaluated and allowed an increase of approximately 170% in the HBsAg concentration (from 0.06 μg/mL to 0.1 μg/mL) (Table 1), suggesting a loss of heterologous gene expression in the absence of selective pressure. This can be explained by a random vector integration into genome, leading to an increase of heterogeneity of the cell population during the cell duplication. With a hygromycin selective pressure only subpopulations containing the vector in active regions of the genome survive, since these subpopulations express the hygromycin resistance gene and consequently the HBsAg heterologous protein.

In this work, HBsAg was expressed by means of simple transfection with an integrating vector in Drosophila S2 cells. The vector used carried a selection gene for hygromycin resistance together with an expression gene for HBsAg, avoiding so the co-transfection procedures. Also in contrast to what was previously described (the use of inductive metalothionein promoter, Deml et al. (1999)) in our study the HBsAg S gene of S2AcHBsAgHy cells was under the control of the constitutive actin promoter and the HBsAg was secreted efficiently into the cell culture supernatant attaining concentrations of up to 10 μg per 1E7 cell. Efficient secretion of HBsAg particles into the cell supernatant was also reported for mammalian cells (Marion et al. 1979; Liu et al. 1982), but not for yeast (Valenzuela et al. 1982; Hitzeman et al. 1983; Janowicz et al. 1991) or Lepidoptera cells upon recombinant baculovirus infection (Lanford et al. 1989). Our findings in terms of HBsAg synthesis were comparable to those reported for Lepidoptera cells using the baculovirus expression system (Kang et al. 1987; Lanford et al. 1989) or mouse cells transfected using a bovine papilloma virus vector system (Hsiung et al. 1984). Our approach allowed us to obtain HBsAg expression levels comparable to those previously published (Deml et al. 1999). In addition, our data show also that HBsAg expression levels were not significantly influenced by the cell culture system and, most important, that a further optimization can be carried out by selecting a culture medium for high production levels. Although the levels of HBsAg attained in animal cell cultures are

much lower than those obtained in yeast cells, which are currently used for vaccine production, stably transfected DS-2 cells provide an attractive alternative source for the production of not only HBsAg but also other viral proteins.

The HBsAg produced by S2AcHBsAgHy cells was reactive in ELISA, suggesting that this protein has antigenicity similar to that of the natural HBsAg found in humans. Drosophila S2 cells combine some advantages for bioprocesses, such as high attainable cell densities, stable gene expression by established cell lines, reduced costs of cell culture media and accumulated knowledge on *Drosophila melanogaster* biology and genetics.

The data presented here indicate that the Drosophila S2 cell system represents a novel and promising approach for heterologous protein expression. Using the HBV S gene as a model, we have shown that constitutive (pAc) or inductive (pMt) (Deml et al. 1999) protein expression in Drosophila cells were similar and efficient. No variation was found in the protein expression performed in different cell culture flasks, suggesting scaling up feasibility. However, the medium used for cell cultivation influenced the magnitude of protein expression, indicating the necessity of further culture medium and protein expression optimization. Moreover, antigenic properties were maintained, pointing out to an efficient system for antigen production. Altogether our data encourage further quantitative and qualitative optimization studies of recombinant proteins.

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